Original article (short paper)

Acute effects of resistance exercise performed on ladder on energy metabolism, stress, and muscle damage in rats

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Abstract — Aim: To evaluate the acute effects of a resistance exercise session performed on ladder on energy metabolism, stress, and muscle damage in rats. Methods: Male Wistar rats were randomly distributed in Exercise (E) (n=30) and Control (C) (n = 20) groups. The E group performed a resistance exercise session on a vertical ladder with weights on their tails. Blood samples were collected at rest and after each climb to analyze lactate levels and ten minutes after the last climb to analyze lactate dehydrogenase (LDH), creatine kinase (CK), and corticosterone levels. Results: Blood lactate levels remained stable during exercise. Serum corticosterone, blood glucose, LDH and CK levels increased and glycogen content decreased in the E group, when compared to the C group. Conclusion: These results suggest that resistance exercise performed on ladder is a model of high-intensity exercise. However, the stabilization of lactate during the session suggests that the aerobic metabolism is an important factor during the intervals between climbs.

Keywords: resistance exercise; metabolism; stress; muscle damage

Introduction

Over the last decade, several studies have described the acute and chronic physiological effects of resistance exercise[1,2]. These findings have established the importance of this modality of exercise to improve physical functioning and quality of life in healthy people[2] or in patients with chronic diseases such as cancer[3] or diabetes[4]. Thus, the American College of Sports Medicine recommends resistance exercise as a primary intervention for health maintenance in young and old people[1] and for prevention and treatment of obesity[5].

Regarding the acute physiological responses of the resistance exercise, it is well known that, during high-intensity activities, the phosphagen system (ATP-PCr) and anaerobic glycolysis, respectively, are crucial to ATP synthesis and maintenance of exercise intensity. Glycolysis generates lactic acid, which breaks down into lactate and hydrogen ions as co-products, and lactate is removed from the active fiber muscles to the blood[6]. The enzymes creatine kinase (CK) and lactate dehydrogenase (LDH) are required in the ATP-PCr and in lactate production, respectively, and both are released into the bloodstream when tissue damage occurs[7,8]. The aerobic system predominates during the interval between sets and exercises, using lipids as main substrate and metabolizing the increased lactate in the heart, liver, and non-working muscles[6].

The glucocorticoids secreted from the adrenal cortex have an important role in the metabolic responses in high-intensity exercises[9]. Corticosterone acts along with the sympathetic nervous system and catecholamines, increasing gluconeogenesis and glycogenolysis[10,11]. Thus, corticosterone blood levels are considered a reliable physiological index of the stress imposed by exercise[10,12].

Different animal models have been used experimentally to understand the mechanisms behind the resistance exercise-induced benefits[13,15]. Among them, stands out the resistance exercise performed on ladder, described by Hornberger and Farrar[16]. The authors showed this specific model mimics many of the chronic physiological adaptations observed in human progressive resistance exercise, increasing the total strength and enhancing myofibrillar protein[16] and muscular glycogen[17]. Additionally, a growing body of evidence has shown that the resistance exercise performed on ladder is also interesting to understand the effects of this exercise modality in the prevention and treatment of chronic diseases, such as obesity and cardiovascular diseases[15,18,19].

However, the acute effects and metabolic characteristics of this particular exercise model have been little explored, and it is still unknown if this model mimics the acute physiological responses of the resistance exercise in humans. Thus, this study aimed to evaluate the effects of a single session of resistance

exercise performed on ladder on lactate, corticosterone blood levels, CK and LDH activity, and glucose and lipid metabolism.

Materials and methods

Animals

A total number of 50 male Wistar rats weighting 340-360g were used. The animals were maintained in collective polypropylene cages (5 animals per cage) with food (MP-77; Primor, São Paulo, Brazil) and water provided *ad libitum*, in a room with controlled temperature (23 ± 2 ºC) and humidity (55 ± 10%). Lights were on from 7:00 am to 7:00 pm. The Ethics Committee for Animal Care and Use of the Federal University of São Carlos approved the experimental protocols used in this study (protocol number CEUA 009/2011), and the latter have been carried out in accordance with the Animal Experimentation Ethical Principles adopted by the Brazilian College of Animal Experimentation (COBEA).

Experimental design

The rats were randomly assigned to two experimental groups: Control (C) (n = 10) and Exercise (E) (n = 20). All animals were familiarized with climbing a ladder for two non-consecutive days. After 72 hours, E and C groups performed the maximum voluntary carrying capacity (MVCC). After two days, the E group performed a single session of resistance exercise and the C group did not perform any type of exercise. The resistance exercise session consisted of four to nine ladder climbs. During the first four ladder climbs, the rats carried 50%, 75%, 90%, and 100% of their previous maximum voluntary carrying capacity (MVCC). During subsequent ladder climbs, an additional 30g load was progressively added until the rat could not climb the entire length of the ladder or complete nine climbs. The rest between sets was 120 seconds. Blood samples (25 µl) were collected from an incision at the tail tip (20) at rest (res); after each climb on the ladder; and at recovery (rec), five minutes after the last climb. Ten minutes after the last climb, another blood sample (200 µl) was collected for analyses of LDH and CK. The blood sample from the C group was collected immediately after collecting the samples from the E group.

Another set of animals was randomly allocated in the C group (n = 10) or E group (n = 10) and performed the same experimental protocol. The animals were euthanized by decapitation at rest (C group) or shortly after acute exercise session (E group) for trunk blood collection in plastic tubes with heparin, to assess blood glucose, glycogen, free fatty acids (FFA), and corticosterone hormone levels. Importantly, a subgroup of animals was used for these analyses to avoid the influence of tail cut.

Resistance exercise

The resistance exercise protocol was adapted from Hornberger and Farrar[16], according to the requirements of this study. The rats were adapted to the resistance exercise (RT) protocol by climbing a vertical ladder (1.1 m; 0.18 m, 2-cm grid, 80° inclination) with a load apparatus without additional weight. The load apparatus was fixed to the tail by wrapping its proximal portion with a self-adhesive foam strip. With the load apparatus fixed to the tail, each rat was placed at the bottom of the ladder and familiarized with the climbing procedure. If necessary, a stimulus with tweezers was applied to the animal's tail to initiate movement. When the rats reached the top of the ladder (house chamber), they were allowed to rest for 120 s. This procedure was repeated until they voluntarily climb the ladder for three consecutive turns without any stimuli.

Each animal performed a test for the evaluation of its MVCC, which consisted of climbs with progressive heavier loads. The initial climb was performed with 75% of the animal's body mass and, after that, an additional 30g weight was added until reaching a load with which the rat could not climb the entire length of the ladder. The highest load that the animal successfully carried through the ladder was considered the MVCC for that training session. Failure was determined when the animal could not move up the ladder after three consecutive stimuli to the tail[21].

Three days after MVCC test, the resistance exercise protocol was performed as described above in the Experimental design.

Lactate analysis

Samples were immediately deposited in tubes containing 50 µl sodium fluoride (1%). To avoid dilution of blood lactate with residual water at the tail of the animal, the rats were quickly dried with a towel immediately before blood collection. The lactate concentrations were determined in a lactate analyzer (YSI model 1500 SPORT; Yellow Springs Instruments Co, Yellow Springs, Ohio, USA), as previously described[20].

Corticosterone, glucose, and free fat acids

Corticosterone was analyzed using a Corticosterone EIA Kit (Cayman Chemical cat. n° 500655, Ann Arbor, MI, USA)[22]. This kit is based on the competition between corticosterone and a corticosterone-acetylcholinesterase conjugate (Corticosterone Tracer) for a restricted number of corticosterone-specific sheep antiserum binding sites. The plates were pre-coated with rabbit anti-sheep IgG and blocked with a proprietary formulation of proteins. The samples were incubated with tracer and antiserum in the wells of the plate for two hours at room temperature on an orbital shaker. The plate was washed to remove any unbound reagents and then Ellman's Reagent was added to the wells. The intensity of the product was determined spectrophotometrically at 405 nm on a Dynex MRX TC Revelation Microplate Reader (Dynex Technologies, Chantilly, VA). Corticosterone analyses were performed in triplicate.

Trunk blood was collected shortly after euthanasia and serum glucose was analyzed using a glucometer device (One Touch UltraMini/Johnson&Johnson, Milpitas, CA, USA).

FFA levels were estimated from 600 µl samples. The samples were incubated in the presence of Co $(NO_3)_2$ and

α-nitroso-β-naftol, following the method described by Novák[23]. Optical densities at 500 nm were measured after fifteen minutes using a spectrophotometer and compared with a standard consisting of 0.4 mM palmitic acid. These analyses were performed in duplicate.

Hepatic and muscular glycogen

To determine glycogen, tissue samples from the liver and soleus and gastrocnemius muscles were dissolved in 10 volumes of 6N KOH in a boiling-water bath for five minutes[15]. The extract was combined with an equal volume of 10% ethanol and one volume of 10% K_2SO_4 was added to precipitate the glycogen. The pellet was resuspended in 3.0 ml of distilled water and glucose was determined using phenol-sulfuric acid[24]. The absorbance was measured at 480nm using a spectrophotometer. These analyses were performed in duplicate.

LDH and CK activity assays

LDH activity was assayed in plasma samples by a spectrophotometric method based on the reduction of pyruvate to lactic acid coupled to NADH oxidation, as previously described[25]. The decrease in absorbance at 340 nm was monitored at 37°C.

CK activity was assayed in plasma samples by a spectrophotometric method based on the phosphorylation of ADP to ATP coupled to the reduction of NADP to NADPH, catalyzed by glucose-6-phosphate dehydrogenase. The increase in absorbance at 340 nm was monitored at 30°C, as previously described[26]. All analyses were performed in duplicate.

Statistical analysis

Values were reported as means ± standard error of the mean (SEM). Data were initially examined for normality using Kolmogorov-Smirnov test and for homoscedasticity using Bartlett criterion. The unpaired Student's t-test was used to compare groups E and C. Lactate data were analyzed using paired Student's t-test. The software package GraphPad Prism 5 (San Diego, CA, USA) was used and values of $P < 0.05$ were considered statistically significant.

Results

Weights, maximal workload, and time to climb

Table 1 shows the body weight and maximal workload in the maximal voluntary carrying capacity (MVCC) test for Control and Exercise groups. No differences were found in these parameters between the groups ($P = 0.8979$ and $P = 0.8283$, respectively). Table 1 also shows the time to climb during the exercise session for the Exercise group.

No differences were found between the groups in liver ($P = 0.2793$), soleus ($P = 0.9432$), and gastrocnemius weights ($P = 0.9999$), as shown in Table 2.

Table 1. Body weight and maximal workload.

	Body Weight (g)	Maximal Workload (g)	Time to climb Exercise session (seconds)
C	351.62 ± 7.75	440.85 ± 12.09	12.39 ± 0.66
E	350.10 ± 8.10	436.95 ± 12.16	-

All values are presented as means ± standard error of the mean; C: control group (n=20); E: exercise group (n=30).

Table 2. Tissue weights from control (C) and exercised (E) groups.

	Liver Weight (g)	Soleus Weight (g)	Gastrocnemius Weight (g)
C	13.62 ± 0.49	0.169 ± 0.07	2.09 ± 0.09
E	12.75 ± 0.56	0.163 ± 0.05	2.09 ± 0.04

All values are presented as means ± standard error of the mean; C: control group (n=20); E: exercise group (n=30).

Lactate

The E group showed an increase ($P < 0.05$) in blood lactate from the second climb (75%) up to recovery, when compared with resting values (RES), as shown in Figure 1.

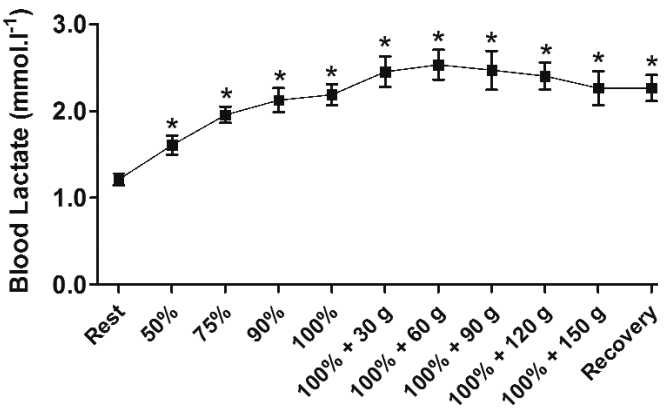

Figure 1. All values are presented as means ± standard error of the mean. Blood lactate concentrations during acute exercise tests are shown for resting conditions (Rest n=20), after climbs carrying 50% (n=30), 75% (n=30), 90% (n=30), 100% (n=30), 100% +30g (n=30), 100%+60g (n=30), 100%+90g (n=24), 100%+120g (n=20), 100%+150g (n=13) of the pre-determined maximum carrying capacity, and following recovery (Recovery n=30). * different from Rest; $P < 0.05$.

Corticosterone, serum glucose, and FFA

The acute exercise increased serum corticosterone concentrations (Figure 2A) in the E group, when compared with the C group (81.43 ± 29.74 ng/ml vs 420.30 ± 19.50 ng/ml; $P < 0.05$).

Regarding serum glucose (Figure 2B), we found an increase when comparing C and E groups (107.9 ± 3.33 vs 142.3 ± 8.43 mg/dL; $P < 0.05$). On the other hand, we did not detect changes in serum FFA (129.46 ± 11.77 vs 170.12 ±15.93 nmol/mL; $P = 0.109$), liver (572.75 ± 47.35 vs 620.50 ± 43.52 nmol/mL; $P = 0.575$), and gastrocnemius (31.08 ± 1.98 vs 37.38 ± 2.90 nmol/mL; $P = 0.219$) FA content.

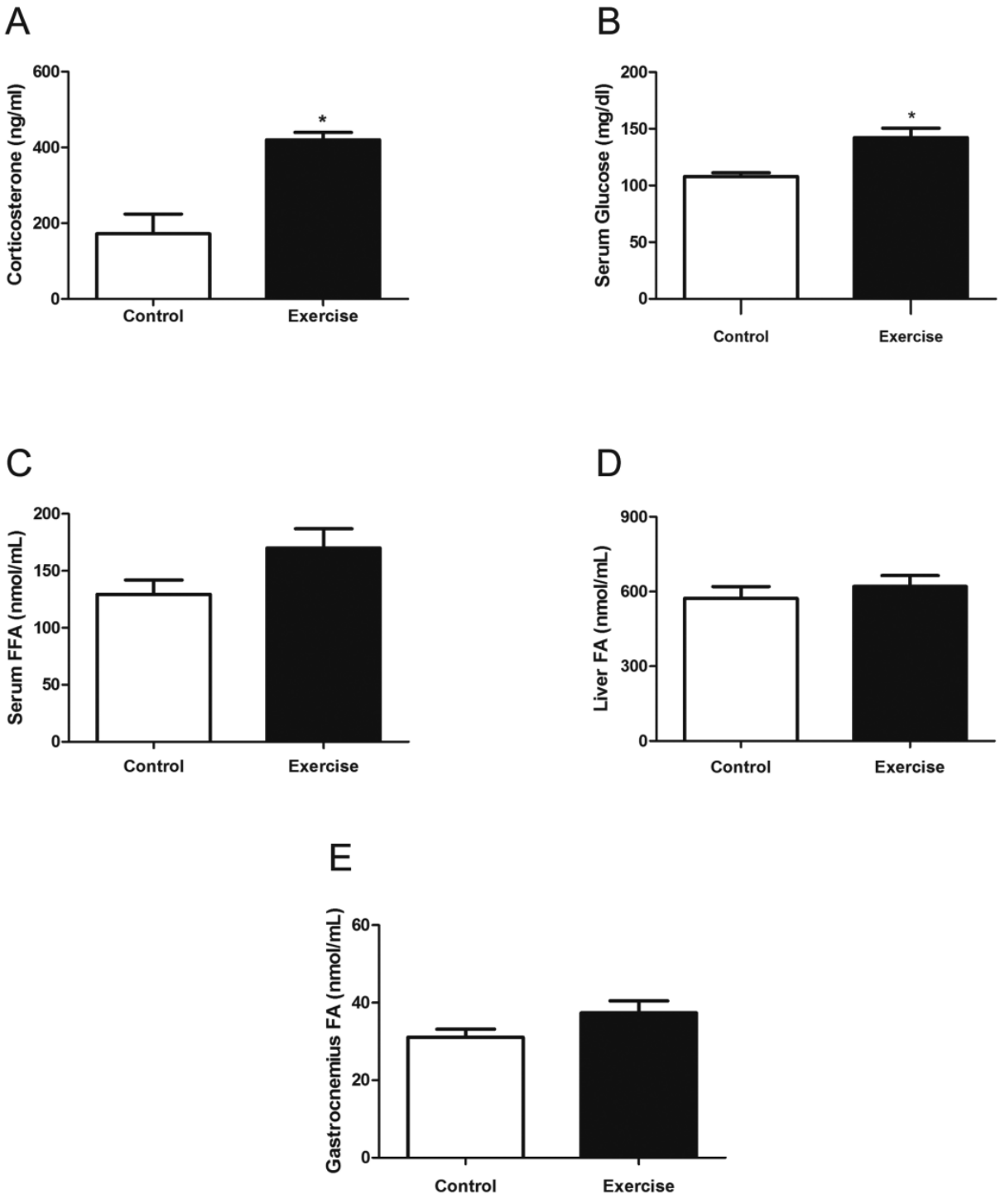

Figure 2. (A) Serum corticosterone concentrations after a single exhaustive resistance exercise session. (B) Serum glucose concentrations after an exercise session. (C) Serum free fatty acid (FFA) concentrations after an exercise session. (D) Liver fatty acid (FA) content after an exercise session. (E) Gastrocnemius FA content after an exercise session. Each bar represents the mean ± SEM of rats. * different from Control group; $P < 0.05$.

Hepatic and muscle glycogen

We also assessed the glycogen content in the liver, observing a decrease after acute exercise in the E group, when compared with the C group (2.15 ± 0.18 vs 3.71 ± 0.29 mg/100g; $P < 0.05$) (Figure 3A). Similarly to the liver, the glycogen content in soleus muscle was reduced after acute exercise in the E group (0.20 ± 0.02 vs 0.45 ± 0.05 mg/100g; $P < 0.05$) (Figure 3B). We found no difference in the glycogen content in gastrocnemius muscle between groups (0.40 ± 0.01 vs 0.42 ± 0.02 mg/100g; $P = 0.556$; Figure 3C).

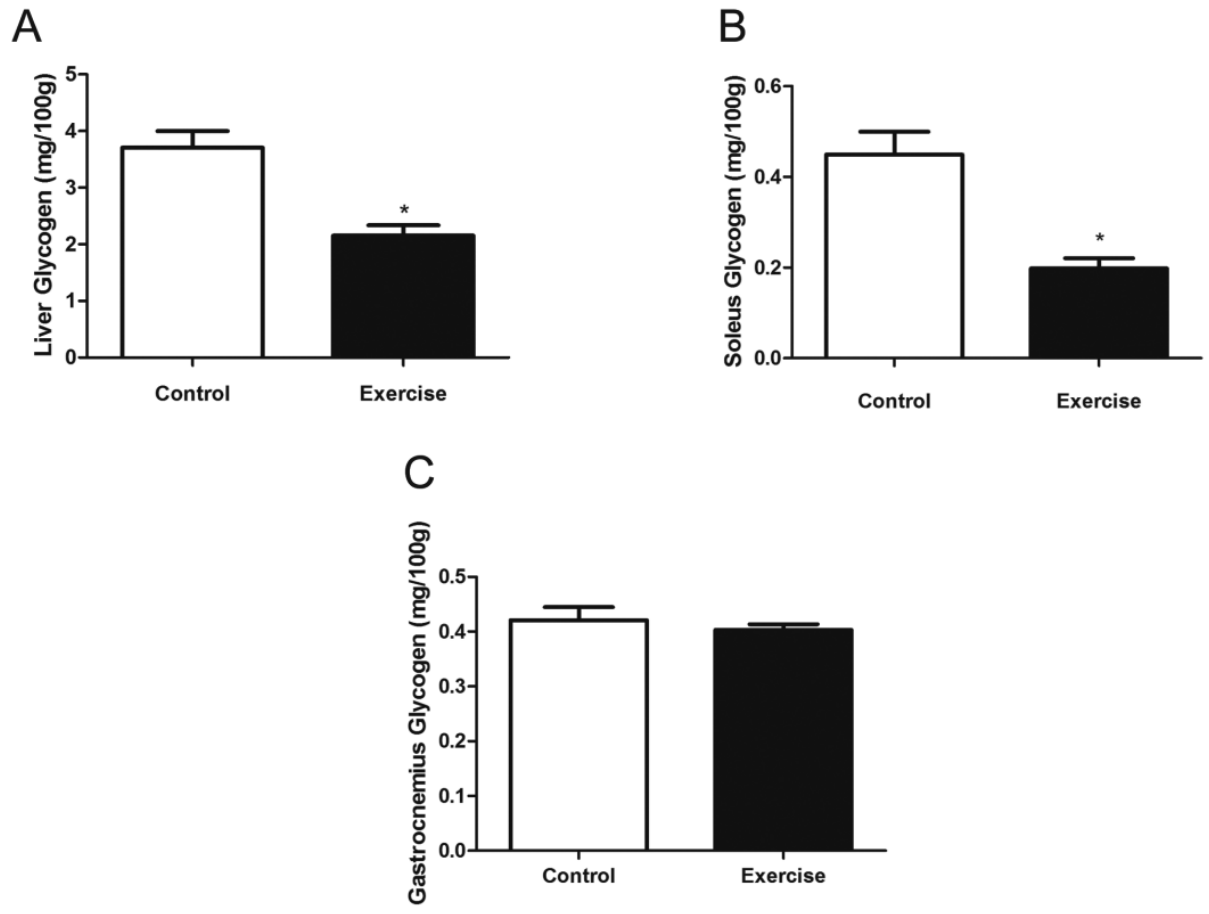

Figure 3. (A) Glycogen concentrations in the liver after a single exhaustive resistance exercise session. (B) Glycogen concentrations in the soleus muscle after a single exhaustive exercise session. (C) Glycogen concentrations in the gastrocnemius muscle after a single exhaustive exercise session. Each bar represents the mean ± SEM of rats. * different from Control group; $P < 0.05$.

Serum LDH and CK

Muscle damage was indirectly estimated by changes in serum LDH and CK activities, as shown in Figure 4A. LDH activity was higher in the E group, when compared with the C group (1,216.3 ± 67.54 vs 810.6 ± 79.07 U/ml; $P < 0.05$), after a single session of resistance exercise.

CK activity was also higher in the E group than in the C group (1,472.2 ± 91.85 vs 1,097.0 ± 133.71 U/ml; $P < 0.05$) (Figure 4B).

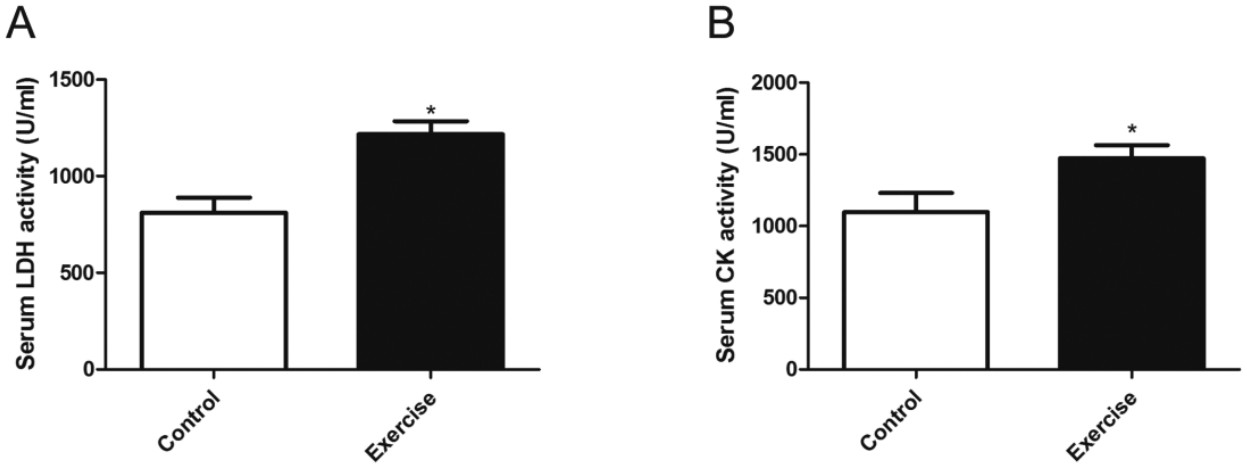

Figure 4. (A) Mean serum lactate dehydrogenase (LDH) activities after a single exhaustive resistance exercise session. (B) Mean serum creatine kinase (CK) activities after a single exhaustive exercise session. Each bar represents the mean ± SEM of rats. * different from Control group; $P < 0.05$.

Discussion

To our knowledge, this study is the first to characterize the acute physiological responses of a resistance exercise performed on ladder. Our main findings are that an acute session of this exercise model increases biomarkers of muscle damage and circulatory stress biomarkers as well as reduces hepatic and muscle glycogen content. Additionally, blood lactate levels remained stable during the resistance exercise session in this particular model.

The resistance exercise increased blood lactate concentrations after the first climb. However, they tended to stabilize during the exercise session, despite the progressive increase in load after each climb. These results suggest a balance between lactate production and lactate elimination[20]. The average time to climb was approximately 12 seconds, which does not allow a significant lactate production, and 120 seconds of recovery between climbs seems to be sufficient for the removal of lactate through oxidation in the heart and skeletal muscles[27]. It is well established that this process is responsible for 70-75% of lactate removal and that the remaining lactate (30-25%) is metabolized through gluconeogenesis (i.e., the Cori cycle) by the liver[28-30]. The present data contrast with a previous study that has shown an exponential rise in blood lactate concentrations in the MVCC test performed on ladder in ovariectomized female rats[31]. Differences by sex in the enzyme activities and energy metabolism during exercise[32,33] might explain these differences.

It was previously demonstrated that a single session of intermittent jump exercise[34] or exhaustive exercise performed on treadmill[35] promote increases in corticosterone plasma levels in rats. These data are consistent with our findings, which showed higher serum corticosterone concentration (~2.4 fold) after a single session of resistance exercise performed on ladder by the E group. Corticosterone secretion is a physiological response to stress, suggesting that the exercise induced changes in the HPA axis[36,37]. Of note, this particular exercise model seems to promote lower relative change in corticosterone levels than a single exercise session of swimming, as previously observed[11]. This difference may be due to the necessity for the animals to keep exercising in the water to avoid drowning[11].

Glucocorticoids have important metabolic functions, including their effects on the metabolism of glucose, lipids, and proteins, inducing increases in blood glucose, mobilization of fatty acids from fat reserves to active tissues[11,36], and rapid mobilization of fat and amino acids from stores for using in both the synthesis of compounds (such as glucose) and as energetic sources[38]. This is in line with our results, which have shown increases in blood glucose concentrations and reduction in liver and soleus glycogen content in the exercise group, when compared with the control group. However, we found no differences in the gastrocnemius contents between the two groups. These results were expected, since it has been shown that the soleus muscle is more recruited during the movement of climb, when compared with the gastrocnemius muscle[16]. This decrease seems to be linked with the glycogenolysis resulting from the sharp increase of sympathetic activity and glucocorticoid hormones induced by exercise[39,40].

There were no differences between groups in serum, liver, and gastrocnemius fatty acid content. These results suggest a predominant use of glycogen in comparison to lipids, consistent with the concept that high-intensity exercises are associated with the depletion of muscle glycogen[40].

In this study, animals that performed a single session of resistance exercise showed higher serum LDH and CK activities. Both enzymes have been used as indirect biomarkers for muscle damage[8,41-43]. Our findings are in line with a previous study that showed increases in LDH and CK activity after a single session of high-intensity exercise performed on treadmill until exhaustion in rats[13]. It is possible that the alterations in these enzymes reflect muscle damage, which could explain in part the skeletal muscle hypertrophy observed in previous studies that used the same exercise protocol[16,44]. On the other hand, another study showed that a single session of high-intensity resistance exercise, which required full range of motion at the ankle, did not promote increases in LDH and CK in rats[45]. Differently from our study, the measurements of LDH and CK were performed after the fifth session and not after the first, as we did in our study. It could explain these discrepancies, since it is well accepted that the adaptation of muscles to the exercise decreases muscle damage[42,46]. For example, it was demonstrated that eccentric resistance exercise in pre-trained individuals did not increase CK activity[42]. Further studies using direct markers for muscle damages, such as morphology, are necessary to confirm muscle damage after a single session of resistance exercise performed on ladder.

Conclusion

In conclusion, we showed that a single session of resistance exercise performed on a ladder increases indirect markers of muscle damage and corticosterone blood levels. Our results thus suggest an increased use of glycogen instead of lipid, consistent with the hypothesis that this is a high-intensity model of exercise. However, the tendency to stabilization of the blood lactate suggests that the aerobic metabolism has a key role during the intervals between climbs. Further studies are necessary to evaluate other variables, including the time-course of muscle damage after a single session and whether adaptation occurs after long-term of training. Furthermore, the impact of different rest intervals between sets on metabolic responses, particularly in lactate concentrations, should be examined.

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